

SEP 27 2006

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Currently Amended) A method for enzymatically producing defined glycosaminoglycan polymers comprising the steps of:

providing at least one functional acceptor, wherein the functional acceptor has at least two sugar units selected from the group consisting of uronic acid[, ] and hexosamine ~~and structural variants or derivatives thereof~~;

providing at least one recombinant glycosaminoglycan transferase having an empty acceptor site and being capable of elongating the at least one functional acceptor in a controlled fashion to form extended glycosaminoglycan molecules, the at least one recombinant glycosaminoglycan transferase selected from the group consisting of:

- (a) a recombinant glycosaminoglycan transferase having an amino acid sequence ~~essentially~~ as set forth in SEQ ID NO:2;
- (b) a recombinant glycosaminoglycan transferase encoded by a nucleotide sequence ~~essentially~~ as set forth in SEQ ID NO:1;
- (c) a truncated form of (a) encoded by a nucleotide sequence ~~essentially~~ as set forth in any of SEQ ID NOS:10, 20, 27-32 and 71;
- (d) a mutated form of (a) encoded by a nucleotide sequence ~~essentially~~ as set forth in any of SEQ ID NOS:11, 12, 16-19, 33-50;

(e) a recombinant glycosaminoglycan transferase encoded by a nucleotide sequence capable of hybridizing to **[a] the nucleotide sequence of SEQ ID NO:1** ~~selected from the group consisting of (b)-(d)~~ under hybridization conditions comprising hybridization at a temperature of ~~about~~ 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, followed with washing in 3x SSC at ~~about~~ 42°C; and providing at least one UDP-sugar selected from the group consisting of UDP-GlcUA, UDP-GlcNAc, **and UDP-GlcN** ~~and structural variants or derivatives thereof~~ in a stoichiometric ratio to the at least one functional acceptor such that the at least one recombinant glycosaminoglycan transferase elongates the at least one functional acceptor to provide glycosaminoglycan polymers wherein the glycosaminoglycan polymers have a desired size distribution such that the glycosaminoglycan polymers are substantially monodisperse in size such that the glycosaminoglycan polymers have a polydispersity value in a range of from 1.0 to 1.2, and wherein the desired size distribution is obtained by controlling the stoichiometric ratio of UDP-sugar to functional acceptor.

2. (Currently Amended) The method of claim 1 wherein, in the step of providing at least one functional acceptor, uronic acid is further defined as a uronic acid selected from the group consisting of GlcUA, iduronic acid (IdoUA), **and** GalUA, ~~and structural variants or derivatives thereof~~.

3. (Currently Amended) The method of claim 1 wherein, in the step of providing at least one functional acceptor, hexosamine is further defined as a

hexosamine selected from the group consisting of GlcNAc, GalNAc, GlcN, and GalN, ~~and structural variants or derivatives thereof.~~

4. (Previously Presented) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is a hyaluronic acid (HA) oligosaccharide having between about three sugar units and about 4.2 kDa.

5. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is an HA polymer having a mass in a range of from about 3.5 kDa to about 2 MDa.

6. (Currently Amended) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is a chondroitin oligosaccharide comprising at least ~~about~~ three sugar units.

7. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is a chondroitin polymer.

8. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is a chondroitin sulfate polymer.

9. (Previously Presented) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is a heparin, heparan or heparosan polymer.

10. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the functional acceptor is an extended acceptor selected from the group consisting of HA chains, chondroitin chains, heparosan chains, mixed glycosaminoglycan chains, analog containing chains, and combinations thereof.

11-12. (Canceled)

13. (Currently Amended) The method of claim 1 wherein, in the step of providing at least one recombinant glycosaminoglycan transferase, the at least one recombinant glycosaminoglycan transferase comprises a recombinant single action glycosyltransferase capable of adding only one sugar unit selected from the group consisting of GlcUA, GlcNAc, ~~or a structural variant or derivative thereof~~ and GlcN.

14. (Canceled)

15. (Currently Amended) The method of claim 1, wherein the at least one recombinant glycosaminoglycan transferase is immobilized and the at least one functional acceptor and the at least one of UDP-GlcUA, UDP-GlcNAc, and UDP-GlcN ~~a structural variant or derivative thereof~~ are in a liquid phase.

16. (Original) The method of claim 1, wherein the at least one functional acceptor is immobilized and the at least one UDP-sugar are in a liquid phase.

17. (Original) The method of claim 1, further comprising the step of providing a divalent metal ion.

18. (Original) The method of claim 17, wherein the divalent metal ion is selected from the group consisting of manganese, magnesium, cobalt, nickel and combinations thereof.

19. (Original) The method of claim 1, wherein the method occurs in a buffer having a pH from about 6 to about 8.

20-22. (Canceled)

23. (Original) The method of claim 1 wherein the substantially monodisperse glycosaminoglycan polymers have a molecular weight in a range of from about 3.5 kDa to about 0.5 MDa.

24. (Original) The method of claim 23 wherein the substantially monodisperse glycosaminoglycan polymers have a polydispersity value in a range of from about 1.0 to about 1.1.

25. (Currently Amended) The method of claim 24 wherein the substantially monodisperse glycosaminoglycan polymers have a ~~polydispersity~~ **polydispersity** value in a range of from about 1.0 to about 1.05.

26. (Original) The method of claim 1 wherein the substantially monodisperse glycosaminoglycan polymers have a molecular weight in a range of from about 0.5 MDa to about 4.5 MDa.

27-28. (Canceled)

29. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor comprises a moiety selected from the group consisting of a fluorescent tag, a radioactive tag, an affinity tag, a detection probe, a medicant, and combinations thereof.

30. (Original) The method of claim 1 wherein, in the step of providing at least one UDP-sugar, at least one UDP-sugar is radioactively labeled.

31. (Previously Presented) The method of claim 1 wherein the glycosaminoglycan polymers are chimeric or hybrid glycosaminoglycans comprising more than one type of polymer backbone.

32. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is a plurality of functional acceptors immobilized on a substrate.

33. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is a plurality of functional acceptors in a liquid phase.

34. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is immobilized on a microtiter plate.

35. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is immobilized on a microarray slide.

36. (Original) The method of claim 1 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is sulfated or is a modified oligosaccharide.

37-73. (Canceled)

74. (Previously Presented) The method of claim 1 wherein the ratio of UDP-sugar to functional acceptor is low to produce products having a molecular weight less than about 0.5 MDa.

75. (Previously Presented) The method of claim 1 wherein the ratio of UDP-sugar to functional acceptor is high to produce products having a molecular weight greater than about 0.5 MDa.

76. (Canceled)

77. (Currently Amended) A method for enzymatically producing defined glycosaminoglycan polymers comprising the steps of:

providing at least one functional acceptor, wherein the functional acceptor is selected from the group consisting of an HA polymer, a chondroitin polymer, a chondroitin sulfate polymer, a heparin, heparan or heparosan polymer, mixed GAG chains, analog containing chains and combinations thereof;

providing at least one recombinant glycosaminoglycan transferase having an empty acceptor site and being capable of elongating the at least one functional acceptor in a controlled fashion to form extended glycosaminoglycan molecules, the at least one recombinant

glycosaminoglycan transferase selected from the group consisting of:

- (a) a recombinant glycosaminoglycan transferase having an amino acid sequence ~~essentially~~ as set forth in SEQ ID NO:2;
- (b) a recombinant glycosaminoglycan transferase encoded by a nucleotide sequence ~~essentially~~ as set forth in SEQ ID NO:1;
- (c) a truncated form of (a) encoded by a nucleotide sequence ~~essentially~~ as set forth in any of SEQ ID NOS:10, 20, 27-32 and 71;
- (d) a mutated form of (a) encoded by a nucleotide sequence ~~essentially~~ as set forth in any of SEQ ID NOS:11, 12, 16-19, 33-50;
- (e) a recombinant glycosaminoglycan transferase encoded by a nucleotide sequence capable of hybridizing to **[a] the** nucleotide sequence **of SEQ ID NO:1** ~~selected from the group consisting of (b)-(d)~~ under hybridization conditions comprising hybridization at a temperature of ~~about~~ 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, followed with washing in 3x SSC at ~~about~~ 42°C; and

providing at least one UDP-sugar selected from the group consisting of UDP-GlcUA, UDP-GlcNAc, and **UDP-GlcN** ~~structural variants or derivatives thereof~~ in a stoichiometric ratio to the at least one functional acceptor such that the at least one recombinant glycosaminoglycan transferase elongates the at least one functional acceptor to provide glycosaminoglycan polymers wherein the glycosaminoglycan polymers have a desired size distribution greater than 1 MDa, and wherein the desired size distribution is



obtained by controlling the stoichiometric ratio of UDP-sugar to functional acceptor.

78. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the functional acceptor is an HA polymer having a mass in a range of from about 3.5 kDa to about 2 MDa.

79-80. (Canceled)

81. (Currently Amended) The method of claim 77 wherein, in the step of providing at least one recombinant glycosaminoglycan transferase, the at least one recombinant glycosaminoglycan transferase comprises a recombinant single action glycosyltransferase capable of adding only one sugar unit selected from the group consisting of GlcUA, GlcNAc and GlcN , ~~or a structural variant or derivative thereof.~~

82. (Canceled)

83. (Original) The method of claim 77, wherein the at least one recombinant glycosaminoglycan transferase is immobilized and the at least one functional acceptor and the at least one UDP-sugar are in a liquid phase.

84. (Original) The method of claim 77, wherein the at least one functional acceptor is immobilized and the at least one UDP-sugar are in a liquid phase.

85. (Original) The method of claim 77, further comprising the step of providing a divalent metal ion.

86. (Original) The method of claim 85, wherein the divalent metal ion is selected from the group consisting of manganese, magnesium, cobalt, nickel and combinations thereof.

87. (Original) The method of claim 77, wherein the method occurs in a buffer having a pH from about 6 to about 8.

88-90. (Canceled)

91. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor comprises a moiety selected from the group consisting of a fluorescent tag, a radioactive tag, an affinity tag, a detection probe, a medicant, and combinations thereof.

92. (Original) The method of claim 77 wherein, in the step of providing at least one UDP-sugar, at least one UDP-sugar is radioactively labeled.

93. (Previously Presented) The method of claim 77 wherein the glycosaminoglycan polymers are chimeric or hybrid glycosaminoglycans comprising more than one type of polymer backbone.

94. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is a plurality of functional acceptors immobilized on a substrate.

95. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is a plurality of functional acceptors in a liquid phase.

96. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is immobilized on a microtiter plate.

97. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is immobilized on a microarray slide.

98. (Original) The method of claim 77 wherein, in the step of providing at least one functional acceptor, the at least one functional acceptor is sulfated or is a modified oligosaccharide.

99. (Previously Presented) The method of claim 77 wherein the ratio of UDP-sugar to functional acceptor is low to produce products having a molecular weight less than about 0.5 MDa.

100. (Previously Presented) The method of claim 77 wherein the ratio of UDP-sugar to functional acceptor is high to produce products having a molecular weight greater than about 0.5 MDa.

101-111. (Canceled)

112. (Previously Presented) The method of claim 1 wherein the substantially monodisperse glycosaminoglycan polymers have a polydispersity value in a range of from about 1.0 to about 1.005.

113. (Previously Presented) The method of claim 77 wherein the substantially monodisperse glycosaminoglycan polymers have a polydispersity value in a range of from about 1.0 to about 1.005.

114. (Currently Amended) A method for enzymatically producing defined glycosaminoglycan polymers comprising the steps of:

providing at least one functional acceptor, wherein the functional acceptor has at least two sugar units selected from the group consisting of uronic acid[, ] and hexosamine ~~and structural variants or derivatives thereof~~;

providing at least one recombinant ~~acid~~ glycosaminoglycan transferase having an empty acceptor site and being capable of elongating the at least one functional acceptor in a controlled fashion to form extended glycosaminoglycan molecules, the at least one recombinant glycosaminoglycan transferase selected from the group consisting of:

- (a) a recombinant glycosaminoglycan transferase having an amino acid sequence ~~essentially~~ as set forth in SEQ ID NO:2;
- (b) a recombinant glycosaminoglycan transferase encoded by a nucleotide sequence ~~essentially~~ as set forth in SEQ ID NO:1;
- (c) a truncated form of (a) encoded by a nucleotide sequence ~~essentially~~ as set forth in any of SEQ ID NOS:10, 20, 27-32 and 71;
- (d) a mutated form of (a) encoded by a nucleotide sequence ~~essentially~~ as set forth in any of SEQ ID NOS:11, 12, 16-19, 33-50;

- (e) a recombinant glycosaminoglycan transferase encoded by a nucleotide sequence capable of hybridizing to [a] ~~the~~ nucleotide sequence **of SEQ ID NO:1** selected from ~~the group consisting of (b)-(d)~~ under hybridization conditions comprising hybridization at a temperature of ~~about~~ 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, followed with washing in 3x SSC at ~~about~~ 42°C; and

providing at least one UDP-sugar selected from the group consisting of UDP-GlcUA, UDP-GlcNAc, and **UDP-GlcN** ~~structural variants or derivatives thereof~~ in a stoichiometric ratio to the at least one functional acceptor such that the at least one recombinant glycosaminoglycan transferase elongates the at least one functional acceptor to provide glycosaminoglycan polymers wherein the glycosaminoglycan polymers have a desired size distribution such that the glycosaminoglycan polymers are substantially monodisperse in size such that the glycosaminoglycan polymers have a polydispersity value in a range of from about 1.0 to about 1.1, and wherein the desired size distribution is obtained by controlling the stoichiometric ratio of UDP-sugar to functional acceptor.

115. (Newly Added) The method of claim 1, wherein the defined glycosaminoglycan polymers so produced are capable of acting as a bioadhesive sealant, a tissue engineering aid, a cell matrix mimetic, a cell behavior or growth modulator, a drug delivery agent, or combinations thereof.